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ANALYSIS OF TOTAL ANTIOXIDANT ABILITY OF PLASMA IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) PATIENTS

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ABSTRACT

The present study was carried out to evaluate the total antioxidant ability of plasma in COPD patients and healthy matched controls. A total of 200 subjects were studied out of which 110 were COPD patients and 90 controls. Total antioxidant ability of plasma was measured by the FRAP (ferric reducing ability of plasma) assay. The significance difference ($p>0.001$) was observed in the FRAP value of COPD patients (392.280±06.602) and controls (765.580±7.571). A significance difference ($p>0.001$) was observed in the mean FRAP value of male, female, smokers and non smokers of COPD patients when compared with controls. The total antioxidant value of plasma was lower in severe COPD patients 364.450±6.383 as compared to the moderate COPD patients 434.020±10.752 and significantly different at ($p>0.001$).

KEYWORDS: Antioxidant, COPD, Plasma, FRAP

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by airway inflammation and irreversible airway obstruction. The most important risk factor for progression of COPD is tobacco smoking (Hakhamaneshi *et al*., 2007). Other risk factors include α 1- antitrypsin deficiency, air pollution, socioeconomic status and lower birth weight (Repine *et al*., 1997). Cigarette smoking is the major environmental determinant of COPD (Rahman *et al*., 2005; Foronjy *et al*., 2006). Cigrette smoke contains an estimated 1014 radicals per puff and about 4700 chemicals that include superoxide radical peroxynitrite, and oxides of nitrogen. Nitric oxide (NO•) is present in concentrations of 500–1000 ppm in cigarette smoke (Pryor *et al*., 1993; Eiserich *et al*., 1997). The oxidative stress in the lungs is increased in smokers by the release of reactive oxygen species from alveolar macrophages and neutrophils (Schaberg *et al*., 1992; Rahman *et al*., 1996; Morrison *et al*., 1999). It is proposed that reactive oxygen species (ROS) is the major cause of cell and tissue damage associated with many lung diseases, including COPD (Ahmad *et al*., 2013).

The most prominent reactive oxygen species of physiological significance are the superoxide anion (O2 •-), hydroxyl radical (OH•), nitric oxide (NO•) and hydrogen peroxide (H_2O_2). There were several evidence which suggest that there is an imbalance between oxidants and antioxidants in the lung and blood in smokers and the patients with COPD (McCusker *et al*., 1990; Kondo *et al*., 1994; Rahman *et al*., 1996). Some recent studies also suggested that an imbalance

between oxidants and antioxidants plays an important role in the pathogenesis of COPD (MacNee, 2000; Langen *et al*., 2003; Rahman *et al*., 2006). In normal conditions, the lungs and the blood are protected by various intracellular and extracellular antioxidants against the harmful effects of the reactive oxidants (Ahmad et al., 2013). The Ferric reducing ability of plasma (FRAP) assay is a fast and novel method for measuring antioxidant power of plasma. The principle of FRAP assay is the Ferric to ferrous ion reduction at low pH and formation of a blue coloured ferrous tripyridyltriazine (TPTZ) complex. FRAP values were obtained by observing the absorbance change at 593 nm in a test reaction mixtures with the solution containing ferrous ions in known concentrations. The present study was aimed to evaluate the antioxidant capacity of plasma with the help of FRAP assay in COPD patients and healthy matched controls.

MATERIALS AND METHODS

Subjects

For the present study, a total of 200 subjects were studied out of which 110 were COPD patients and 90 controls matched with respect to age, lifestyle and socioeconomic status. COPD was diagnosed by register medical practitioner, using clinical history, physical examination, and confirmation by airflow obstruction with spirometry test. Airflow obstruction is defined as a ratio of forced expiratory volume in one second (FEV1) to forced vital capacity (FVC) below 70% of predicted value. Severity of the disease measured according to the Global Initiative for Chronic Obstructive Lung Disease guidelines (2006). The study was carried out in Human Genetics Laboratory, Department of Zoology, Kurukshetra University, during the period May-Septemer, 2015. A detailed questionnaire was filled by COPD patients and controls to collect details about the subjects regarding their sex, age and smoking habits. Ethical clearance was obtained from Institutional Ethics Committee, Kurukshetra University, Kurukshetra (No.IEC/14/371) dated-October 1, 2014 for the present study. An informed consent was taken from each subject prior to blood sampling.

Sample Collection and Laboratory Analysis

Blood samples were collected from the vein of the subjects by a registered medical practitioner and taken to the laboratory in K_2 EDTA coated vials (Becton Dickinson) and centrifuged at 2500 rpm. Analysis was done within 12 hours. Total antioxidant ability of plasma was measured by the FRAP (ferric reducing ability of plasma) assay. 100 mL of plasma was mixed with 300 mL distilled water and 3 mL of working FRAP reagent, freshly prepared by adding 10:1:1 ratio of 300 mmol/L acetate buffer, 10 mmol/L 2, 4, 6-tripy ridyl-S-triazine (HIMEDIA) in 40 mmol/L HCl and 20 mmol/L $FeCl_3 \times 6H_2O$ (HIMEDIA). Ascorbic acid was taken as standard. Absorbance was measured at 593 nm at zero minute after vortexing. After that, samples were placed in a water bath at 37 ˚C and absorbance was taken after 4 minutes.

RESULTS

The general and clinical characteristics of the subjects are given in the Table1. The average age of COPD patients was 56.736 years and that of control subjects 53.667 years. The significance difference ($p>0.001$) was observed in the FRAP value of COPD patients 392.280±06.602 and controls 765.580±7.571. A significant difference ($p>0.001$) was observed in the mean FRAP value of male, female, smokers and non smokers of COPD patients when compared with controls Fig1. COPD patients were categorized into moderate and severe according to the severity of the disease. Marked significant difference was observed in the spirometry parameters i.e. FEV (%Predicted), FEV1 (%Predicted) and FEV1/FVC (%Predicted) of severe COPD patients when compared with moderate COPD patients. The total antioxidant

value of plasma was lower in severe COPD patients 364.450±6.383 as compared to the moderate COPD patients 434.020±10.752 and significantly different at (p>0.001) (Table 2).

Statistical Analysis

Statistical analysis was carried out using software SPSS version 16 and the results were evaluated by Student's t test.

Table I: General and Clinical Characteristics of the Subjects

Characterstics	COPD	Controls
N	110	90
Age (years)	56.736±1.210	53.667±1.613
Sex(male/female)	77/33	63/27
Smokers/Non smokers	49/61	31/59
FRAP (µmol/ml)	392.280±06.602	765.580±7.571**

****Significant (p>0.001) unpaired Student's t test.**

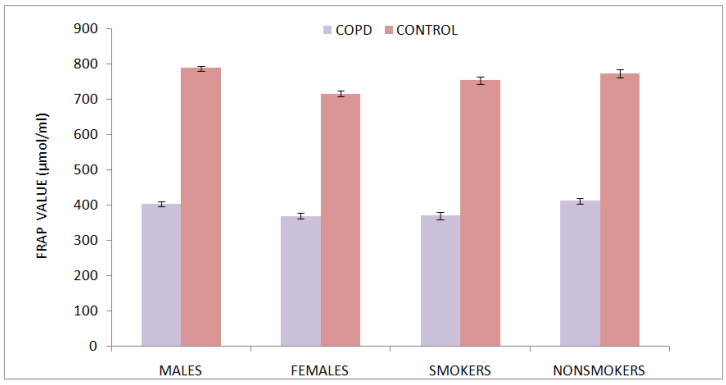

Figure 1: Ferric Reducing Antioxidant Power of Plasma (µmol/Ml) in COPD Patients and Controls

Table 2: Comparision of Different Spirometry Parameters and FRAP Values in Moderate and Severe COPD Patients

Charecterstics	Severity of Disease		P VALUE
	Moderate (44)	Severe (66)	
FEV1(%PREDICTED)**	57.273±0.715	36.409±0.482	0.000
FVC(%PREDICTED)**	63.068±1.970	55.045±1.307	0.001
FEV1/FVC(%PREDICTED)**	78.727±1.709	64.470±0.666	0.000
FRAP VALUE (µmol/ml)**	434.020±10.752	364.450±6.383	0.000

****Significant (p>0.001) unpaired Student's t test.**

DISCUSSIONS

Cigarette smoke contains extremely high concentration of oxidants. Various recent studies have investigated the role of reactive oxygen species in the production of the inflammatory response in the central and the peripheral airways of COPD patients (MacNee et al., 2001; Saetta et al., 2001). Skeletal muscles generated free radicals at rest in COPD patients and their production increases during contractile activity. Increased production of free radicals leads to oxidant–antioxidant imbalance that causes oxidative stress (Heunks et al., 2000). Oxidative stress is suggested to play a crucial role in the pathophysiology of the COPD (Rahman *et al*., 2002). Several methods (Ghiselli et al., 1985; Wayner *et al*., 1985; Glazer, 1990; Whitehead et al., 1992; Cao G et al., 1993; Miller et al., 1993; Cao G et al., 1995; Benzie and Strain, 1996;) have

been developed to analyse the total antioxidant ability of plasma and serum but we have used FRAP assay because it is very convenient fast and inexpensive in terms of operation. In the present study we have observed an decreased in total antioxidant capacity of plasma in the patients with COPD as compared to controls. Nadeem *et al* (2005), Emin *et al* (2010) and Ahmad *et al.* (2013) have also reported the lower value of FRAP in COPD patients than controls.The present results contradict the data of Hakhamaneshi *et a*l. (2007) who found about two fold increase in the values of FRAP in COPD patients as compared with the controls. The present study also observed a lower antioxidant ability of plasma in males, females, smokers and non smokers of COPD patients as compared with controls. A significant decrease in FRAP values of COPD patients who have severe COPD than moderate was observed during the study. Nadeem *et al*. (2005) and Ahmad *et al.* (2013) have also noticed lower values of FRAP in severe COPD patients as compared to the moderate COPD patients. Several recent studies have reported the analogous effect of smoking on total antioxidant capacity either in patients or control individuals (Rahman et al., 1996, 2000; Ceylan, et al., 2007). Our results also showed that smoking significantly affected the total antioxidant ability of plasma in COPD patients.

CONCLUSIONS

The present study suggested that COPD patients have significantly lower value of plasma as compared to the controls we have also observed increased oxidative stress in severe COPD patients as compared to moderate COPD patients.

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